भारतीय मानक
Indian Standard

IS 17085 : 2019

वस्त्र रंजक सामग्री — रूबिया (मद्दार) — पहचान की विधि

Textile Dyestuffs — Rubia (Maddar) — Identification

ICS 59.040

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भारतीय मानक ब्यूरो
BUREAU OF INDIAN STANDARDS
मानक भवन, 9 बहादुरशाह ज़फर मार्ग, नई दिल्ली – 110002
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002
www.bis.gov.in www.standardsbis.in

January 2019 **Price Group 7**

Textile Speciality Chemicals and Dyestuffs Sectional Committee, TXD 07

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by Textile Speciality Chemicals and Dyestuffs Sectional Committee had been approved by the Textile Division Council.

The revival of natural dyes has been for two reasons:

a) Due to the harmful effect of synthetic dyes globally safer natural dyes are being preferred, and

b) Ban of azo dyes has also brought back natural dyes in limelight. Natural dyes are being used extensively for dyeing of cotton, silk and wool substrate.

Thus, *Rubia* dye (Maddar) has its own characteristics of being a natural dyestuff and its method of use in dyeing and printing these textile substrates. In order to achieve optimum quality, with minimum lot to lot variation and variations in dyeing due to same dye of different manufacturers, it is essential to standardize this natural dyestuff. This will also lead to minimize cost benefit ratio and help the user to adjust his dyeing or printing recipes suitably.

Rubia cordifolia produces reddish orange dyes which has been used for dyeing textiles since ancient times. The botanical name of Manjistha is known as Indian *Rubia*, it belongs to family *Rubia*ceae. It grows throughout India, in hilly districts up to 3 500 meters height. Generally, this plant is found on the Himalayan side of India. It is a perennial, herbaceous climber. It is a good source of anthraquinone, reddish orange dyes which is obtained from roots, stem and leaves of the plant. It has been proved to be good and fast dye for fabrics.

Stem and roots of *Rubia cordifolia* are used as natural dye as they contain reddish orange colorant. *Rubia* dye (Indian Maddar) is used for producing bright red shades on cotton, silk and wool with all round fastness properties. This is a non-dischargeable natural dyestuff, but is suitable for direct printing as well.

It is for the first time that the standard for natural dyestuff *Rubia* dye (Maddar) has been written, it is mainly for the ease of buyers, seller and users of this natural dyestuff. Standardization and test method will bring all the major parameters under scan and match. The main intention of this specification is to identify the natural dyestuff from the synthetic analogue which is usually used as adulterant for cost modification and color enhancement purposes.

The composition of the Committee responsible for the formulation of this standard is given at Annex H.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

TEXTILE DYESTUFFS —
RUBIA (MADDAR) — IDENTIFICATION

1 SCOPE

This standard prescribes test method and identification requirement of dried/aqueous extract of *Rubia* dye (Maddar) dye and Rubia dyed textiles. The detection and identification of synthetic alizarin and natural colorant madder are conducted using kit method given in Annex A. Confirmatory tests can be carried out through Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC) and High Performance Liquid Chromatography (HPLC) by chromatographic techniques as given in Annex B, C and D as well as by UV-Visible and Fourier Transform Infrared Spectroscopy (FTIR) spectroscopic techniques as given in Annex E and F.

2 REFERENCES

The following standard contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the edition indicated were valid. All standard is subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standards indicated below.

IS No.	*Title*
1070 : 1992	Reagent grade water

3 TERMS AND DEFINITIONS

For the purposes of this standard, the following terms and definitions shall apply.

3.1 Maddar (*Rubia cordifolia* and *Rubia tinctoria***)**

3.1.1 Stem and roots of *Rubia cordifolia* (Indian Maddar). *Rubia tinctoria* (European Maddar) is another species which also belongs to Rubeacea family. Theses anthraquinone dyes are used as natural dye as they contain reddish orange colorant. *Rubia* dye (Indian Maddar) is used for producing bright red shades on cotton, silk and wool with all round fastness properties. Structurally the Rubia dye consists of natural alizarin, manjistin, purpurin and other anthraquinone moieties.

3.1.2 *Rubia* dye (Maddar) has its own characteristics of being a natural dyestuff and its method of use in dyeing and printing these fabrics and yarns. In order to achieve optimum quality with minimum lot to lot variation and variations in dyeing due to same dye of different manufacturers, it is essential to standardize and identify this natural dyestuff.

3.1.3 *Rubia* grows throughout India, in hilly districts upto 3 500 meters height. Generally, this plant is found on the Himalayan side of India. It is a perennial, herbaceous climber. It is a good source of anthraquinone, reddish orange dyes which is obtained from roots, stem and leaves of the plant. It has been proved to be good and fast dye for fabrics.

3.1.4 Similarly *Rubia tinctoria* (European Maddar) is another species which also belongs to anthraquinone dyes and has similar color content.

3.2 Natural Colour — Colorants from natural origin, particularly materials obtained from plant parts:

a) Stem,
b) Root, and
c) Leaves.

Extracted without any chemical reaction primarily used in textiles for dyeing or printing.

4 PRINCIPLE

Natural colorants are usually never a single compound, they are composed of several compounds which are structurally similar, each of them having their characteristic bonding with the fabric based on their structure uniqueness. Their contribution in the total colorant may vary. Therefore, the analysis of these individual components can be carried out by chromatographic and spectroscopic techniques. The authenticity of the natural colorant can be established by these techniques very clearly. Thus natural colorant madder can be easily differentiated from synthetic alizarin.

NOTE — Alizarin is molecule from synthetic origin as well as it is also present in natural madder colorant. However, very discrete differences in the chromatograms and spectra of the two can be observed.

5 PROCEDURE

5.1 Preparation of Natural Colorant Sample

Stock solution of natural colorant madder and synthetic alizarin are prepared in methanol containing 1.000 mg/l. These solutions are further used after dilution if necessary, for chromatographic and spectroscopic analyses.

5.2 Analysis

The detection and identification of synthetic alizarin and natural colorant madder are conducted using kit method given in Annex A, TLC, HPTLC and HPLC by chromatographic techniques as given in Annex B, C and D as well as by UV-Visible and FTIR spectroscopic techniques as given in Annex E and F. The method for extraction of natural rubia dye from dyed swatches is given in Annex G.

5.3 Qualification and Identification of Alizarin

Comparison between analyses of standard synthetic alizarin and natural colorant madder dyes and their dyed fabrics through **5.3** could show the results of discrete differences in standard and madder sample. The specific tests to be conducted are given in Table 1.

6 REPORT

The report shall include the following information:

a) Name of the natural dye tested;
b) Test method used; and
c) Identification of the natural source.

7 CHECK OF THE ANALYTICAL SYSTEM

A check of the procedure of analysis and instrument/equipment is also essential. HPTLC, HPLC, UV-Visible spectrophotometer, FT-IR spectrometer and NMR spectrometer must be checked before the analysis of the sample.

8 VALIDATION OF THE METHOD

Method Vvlidation and calibration is carried out using authentic and standard natural dye for UV-Visible spectrophotometric analysis.

a) Reliability of the method,
b) Repeatability, and
c) Comparability.

Table 1 Specific Tests Recommended for Identification of Natural Maddar

(*Clause* 5.3)

Sl No. (1)	Test (2)	Type of Test (3)	Annex No. (4)
i)	Kit test for identification of natural maddar	Preliminary test	Annex A
ii)	Chromatography tests		
	a) Thin Layer Chromatography (TLC)	Mandatory test	Annex B
	b) High Performance Thin Layer Chromatography (HPTLC)	Optional test*	Annex C (*See* NOTE)
	c) High Performance Liquid Chromatography (HPLC)	Optional test *	Annex D (*See* NOTE)
iii)	Spectroscopy tests		
	a) UV-Visible Spectroscopy	Confirmatory test	Annex E
	b) FT-IR Spectroscopy	Optional test*	Annex F (*See* NOTE)

NOTE — HPTLC, HPLC and FT-IR may be done instrumentally, wherever facilities are available.

ANNEX A

(*Clause* 1 *and* 5.3)

KIT TEST FOR IDENTIFICATION OF *RUBIA* DYE (MADDAR)

A-1 PRINCIPLE

Each natural dye has its characteristic molecular composition which is the distinctive feature of that dyestuff. Dissolution of the two samples of *Rubia cordifolia* and *Rubia tinctoria* under the class of maddar dyes in different polarity solvents would bring out the difference and distinction when compared with alizarin. The *Rubia* dye (maddar) bearing different contaminants dissolves in water layer better, whereas, the purer synthetic alizarin dissolves well in dichloromethane solvent.

A-2 APPARATUS

A-2.1 Test Tubes

A-2.2 Test Tube Holder

A-3 REAGENTS

A-3.1 Water

A-3.2 Sodium Hydroxide (NaOH)

A-3.3 Hydrogen Peroxide (H_2O_2)

A-3.4 Ethylene Diamine Tetra Acetic Acid (EDTA) Solution

A-3.5 Potassium Permanganate ($KMnO_4$)

A-3.6 Rubia Dye

A-3.7 Alizarin

A-4 PROCEDURE

Dissolve *Rubia* dye and Alizarin samples (2-5 mg) were in water (10 ml) and make the pH (10.6) alkaline by using 10 percent solution of sodium hydroxide (NaOH), to this, add 5 drops of hydrogen peroxide (H_2O_2), the solution will decolorize and then add 2 ml of 4 percent ethylene diamine tetra acetic acid (EDTA) solution and 2 percent of potassium permanganate ($KMnO_4$) solution one after the other and the mixture was shaken. Yellow color will develop in the solution having natural *Rubia* (test tube –A) while the synthetic alizarin (test tube-B) dye remains unchanged in color.

A-5 CONCLUSION

The clear difference in color identifies the natural *Rubia* (*Rubia*) dye and synthetic alizarin dye very distinctively as shown in Fig. 1.

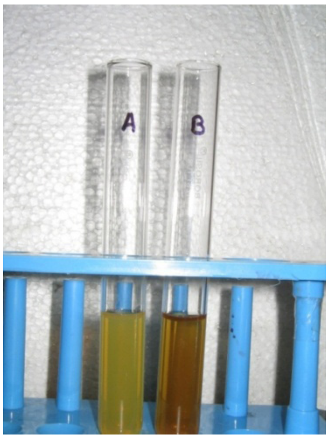

FIG.1 CHANGE ON COLOR FOR *RUBIA* DYE

ANNEX B

(*Clause* 1 *and* 5.3)

CHROMATOGRAPHIC ANALYSIS THIN LAYER CHROMATOGRAPHY

B-1 PRINCIPLE

TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate). Natural *Rubia* dye and synthetic alizarin dyes are compared on TLC.

B-2 APPARATUS

B-2.1 TLC Plates

B-2.2 TLC Elution Chamber

B-2.3 TLC Iodine Chamber

B-2.4 Electronic Balance, Resolution at 0.001 g

B-2.5 Spatula

B-2.6 Sonicator, to be set up at (45 ± 2)ºC

B-2.7 Measuring Cylinder

B-3 REAGENTS

B-3.1 Natural Rubia Dye

B-3.2 Synthetic Indigo Dye

B-3.3 Ethyl Acetate or Dichloromethane

B-3.4 Hexane

B-3.5 Dichloromethane

B-4 PROCEDURE

Prepare *Rubia* dye sample (0.01 gm of maddar sample in 20 ml of solvent) in solvents such as ethyl acetate or dichloromethane. Agitate the prepared sample in sonicator, a mechanical agitating device used for dissolution of maddar dye molecules) for 5 min for complete dissolution of the *Rubia* samples. The solution is then spotted on silica gel coated plate for elution. TLC plates to be eluted using 60 percent ethyl acetate (EtOAc)/40 percent hexane as suitable eluent system for red dyes. The spots are visualized in visible light as well as in iodine chamber. The possible constituents of the extracts are identified by comparing TLC data, that is, color of the spot and R_f values of natural *Rubia* and synthetic alizarin dye.

B-5 OBSERVATION

Rf on the TLC Plate	**In Synthetic Alizarin**	**In Natural Rubia**	**Comment (Describing Difference with Reason)**
60 Percent EtOAc in Hexane	R_f 0.60	R_f 0.60 and 0.20	Presence of two spots in *Rubia* is because of alizarin and Purpurin

B-6 RESULT

Color of the spot and R_f values of natural *Rubia* (RC) and synthetic red (Alizarin) are very different, *Rubia* showing one spot which is fairly polar as shown in Fig. 2.

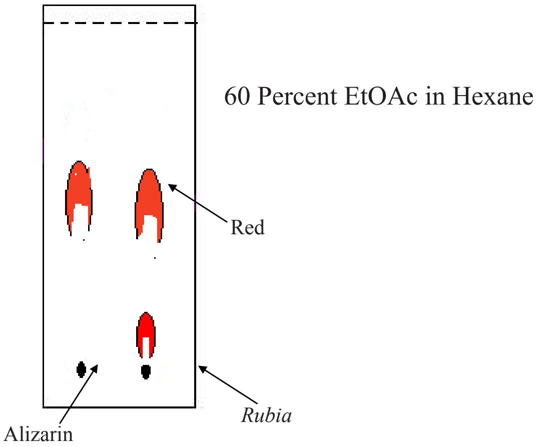

FIG. 2 TLC OF THE TWO DYES

ANNEX C

(*Clause* 1 *and* 5.3)

ANALYSIS OF NATURAL MADDAR COLORANT BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPH (HPTLC)

C-1 PRINCIPLE

HPTLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC precoated plate). Natural *Rubia* dye and synthetic alizarin dye are compared on HPTLC.

C-2 APPARATUS

C-2.1 Imaging System

C-2.2 Densitometer

C-2.3 Sonicator, to be set at (45 ± 2)°C

C-2.4 Measuring Cylinder

C-2.5 Electronic Balance, resolution at 0.001g

C-2.6 Spatula

C-2.7 Filter Paper

C-3 REAGENTS

C-3.1 Natural Rubia Dye

C-3.2 Synthetic Aizarin

C-3.3 Light Petroleum Ether

C-3.4 Ethanol

C-3.5 Methanol

C-3.6 Ethyl Acetate

C-3.7 Formic Acid

C-3.8 Hexane

C-3.9 Chloroform

C-3.10 Precoated Silica Gel Plates HPTLC Silica Gel 60 F254

C-3.11 Natural Product Reagent A (NPR A).

C-4 PROCEDURE

C-4.1 Spotting of the Plates

Use HPTLC applicator for loading the dyes onto the silica gel plates. Apply 40µl samples in the case of Rc and Rt while 20µl for synthetic alizarin.

C-4.2 Method

Prepare *Rubia cordifolia* (Rc) and *Rubia tinctorial* (Rt) as 0.2 percent solution in ethanol, sonicated for 2 min and then filter through filter paper and use for analysis. Take alizarin (Al) 0.1 percent solution in ethanol, sonicated for 2 min and filtered through filter paper and use for analysis.

a) **Stationary phase** : Precoated HPTLC silica gel 60 F254 plates (Size 20 cm X 10 cm, 10 cm X 10 cm).
b) **Mobile phase I** : Light petroleum ether : Ethyl acetate : Methanol (7.5 ml: 2.5 ml : 120 µl).
c) **Mobile phase II** : Light petroleum ether : ethyl acetate : formic acid (7.5 ml : 2.5 ml : 100 µl).
d) **Development** : Saturated chamber.
e) **Chamber saturation**: 10 min.
f) **Development distance**: 80 mm.

Finally, the plates to be documented by the imaging system; developed chromatogram of the same detected by the densitometer with a light beam of visible light and UV ranges of 254 and 366 nm results in very distinctive features. As an additional measure to highlight the bands and their intensity, derivatization with a reagent: Natural Product Reagent A (NPR A) can also be used. It is analyzed by the method described below.

The chromatographic plate is sprayed by 10 percent NPR A reagent and then the plate is dried. The colors of the bands are compared before and after the spray.

C-5 OBSERVATION

C-5.1 All the three samples (Rc, Rt and Al) will show distinct bands shown in Fig. 3, 4 and 5. Fluorescent bands common to Rc and Rt are observed at the spotting area as well as a blue band at Rf 0.5, seems to be very characteristic of maddar dyes (Fig. 3).

C-5.2 Rc shows 9 apparent bands (Fig. 6) while Rt shows 10 apparent bands of different intensities as shown in Fig. 7, while Al shows only 1 broad band (Fig. 5).

C-5.3 Disappearance of bright orange band in Rt after derivatization with NPR A as shown in Fig. 6. Another blue band appears in second run of the plate in solvent system with formic acid system as shown in Fig. 4.

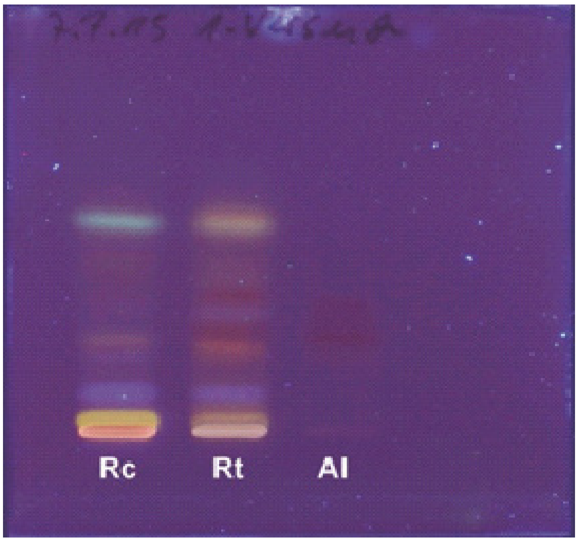

Fig. 3 Rc, Rt and Al run in solvent system 2
First run

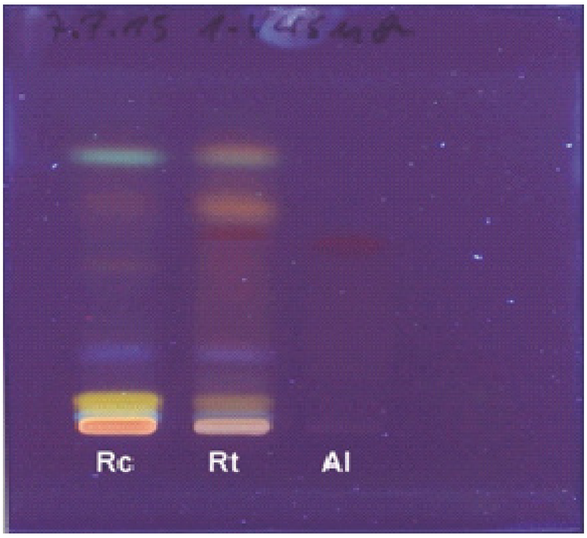

Fig. 4 Rc, Rt, and Al run in solvent system 2,
Double run

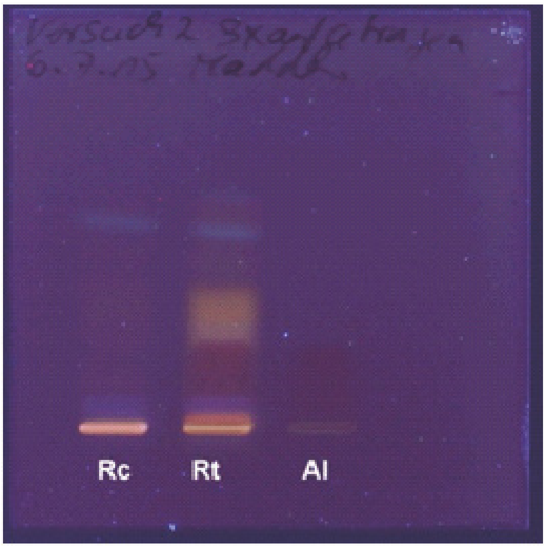

Fig. 5 Rc, Rt and Al Run in Solvent System 1
Double Run

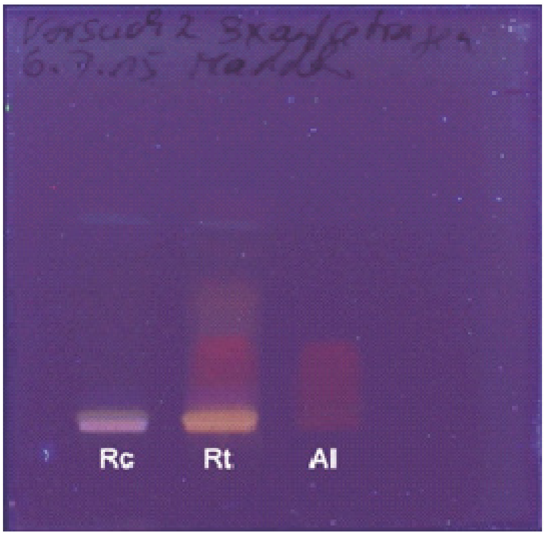

Fig. 6 Rc, Rt, And Al Run in Solvent System 1,
Double Run with NPR A Spray

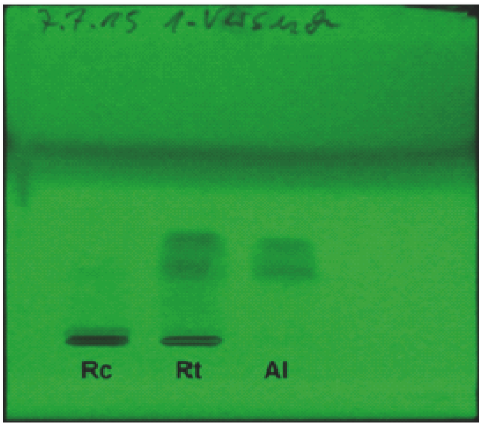

Fig. 7 Plate as in Fig. 3 Under UV Illumination
254 nm

C-5.4 Double run of the plates in the solvent system light petroleum ether: ethyl acetate: formic acid causes very good separation of the components.

C-6 RESULT

Rf of reddish spot in Rt under 366 nm illuminations corresponding with Al's reddish spot will be slightly different as shown in Fig. 3. The difference can also be observed under 254 nm illuminations as shown in Fig. 5. Rt shows many spots of different colours and intensities. The intensity of the reddish spot corresponding to Al is also different in Rc and Rt in Rf as well.

ANNEX D

(*Clause* 1 *and* 5.3)

IDENTIFICATION OF NATURAL *RUBIA* DYE WITH SYNTHETIC ALIZARIN BY HPLC METHOD

D-1 PRINCIPLE

D-1.1 Identification of compounds by HPLC is a crucial part of separation of peaks. In order to identify any compound by HPLC a detector must first be selected. UV detector was selected and was set to optimal detection settings, a separation assay was developed. The parameters of this assay were made to be such that a clean peak of the both the samples are observed from the chromatograph.

D-1.2 HPLC is also used to support the identity of a compound in a mixture when the R_t of a compound is compared with the R_t of a known compound traversing through a reverse phase column. Natural *Rubia* dye and synthetic alizarin dyes are compared on HPLC by making separate run of each under similar conditions.

D-2 APPARATUS

D-2.1 HPLC Machine

D-2.2 Diode Array Detector

D-2.3 C-18 Reverse Phase Column

D-2.4 Electronic Balance, Resolution at 0.001g

D-2.5 Spatula

D-2.6 Micro Syringe

D-3 REAGENTS

D-3.1 Methanol

D-3.2 Ethyl Acetate

D-3.3 Natural Rubia Dye

D-3.4 Synthetic Alizarin Dye

D-4 SAMPLE PREPARATION

Weigh separately the two red dye samples (0.1gm) in 1 000 ml of methanol and inject 1 µL of the prepared solution to the C-18 reverse phase column and eluted through column. The base line shows response within a run of 15 min for natural *Rubia* and synthetic alizarin. The parameters of this assay are made to be such that a clean peak of the both the samples are observed from the chromatograph.

The experimental conditions are as follows:

Eluent: Methanol: Deionised water (90: 10), Isocratic

Stationary phase: Column C18, 150 × 4.6 mm;

Flow rate: 1.0 ml/min;

Detection wavelengths: 255 nm (band width 16 nm),

Pump Pressure: 15 MPa.

Clear observation can be made from the two red dye samples as can be seen in the two chromatographs given below. For synthetic alizarin, one main peak is at 1.6 min and no peak after 3.5 min, while natural *Rubia* has main peak at 1.8 min and a characteristic peak at 11.3 min as shown in Fig. 8 and Fig. 9.

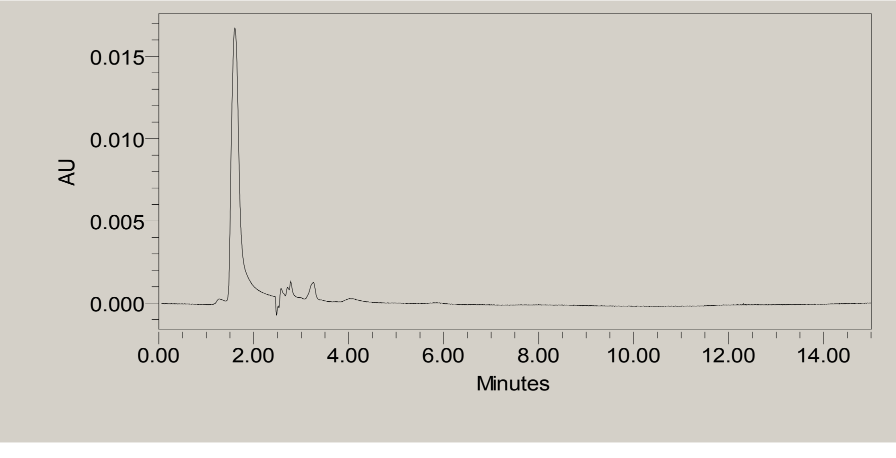

FIG. 8 CHROMATOGRAM OF SYNTHETIC ALIZARIN

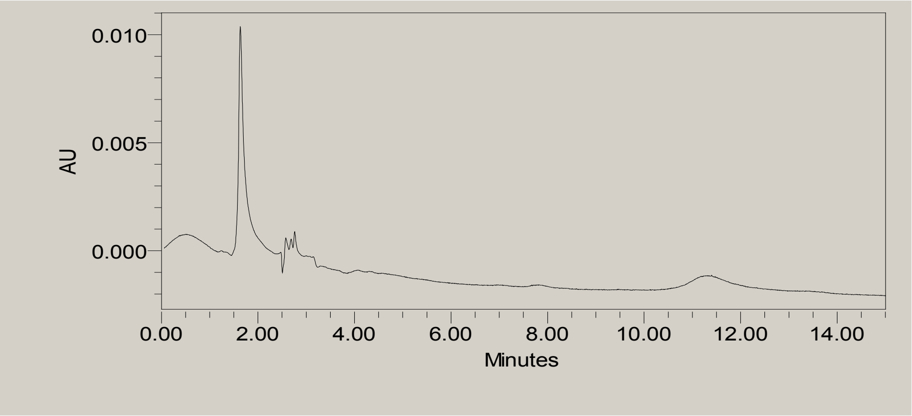

FIG. 9 CHROMATOGRAM OF NATURAL RUBIA

D-5 OBSERVATION

Peak Reading received at (nm)	In Synthetic Alizarin	In Natural Rubia	Comment (Describing Difference with Reason)
255 nm	One main peak is at 1.6 min and no peak after 3.5 min	1.8 min main peak, small peaks at 2.5 and 11.3 min	One main peak is at 1.6 min and no peak after 3.5 min while natural *Rubia* has main peak at 1.8 min and a characteristic peak at 11.3 min

D-6 RESULT

Natural *Rubia* and synthetic alizarin show clear differences in their characteristic peaks and their retention times and their peak heights. Natural *Rubia* will always be showing two equivalent peaks for as compared to synthetic alizarin which shows three peaks due to unpurified sample of the latter, this is a mark of identification between natural *Rubia* and synthetic alizarin.

ANNEX E

(*Clause* 1 *and* 5.3)

IDENTIFICATION OF NATURAL *RUBIA* DYE AND ALIZARIN BY UV –VIS SPECTROSCOPIC METHOD

E-1 PRINCIPLE

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (A) versus wavelength.

Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the path length, b, and the concentration, c, of the absorbing species. *Beer's Law* states that:

$$A = \varepsilon bc$$

Where, ε is a constant of proportionality, called the *absorbtivity*.

Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule. Thus the absorption spectra (optical density) of a particular dye at a particular wavelength is maximum.

E-2 APPARATUS

E-2.1 Ultra Violet — Visible spectrophotometer machine.

E-2.2 Quartz Sample Tube (Cuvette) — Having 1.00 cm light path.

E-2.3 Electronic Balance — Resolution at 0.001g.

E-2.4 Spatula

E-2.5 Whatmann Filter Paper — Grade 42.

E-3 REAGENT

E-3.1 Natural Rubia Dye

E-3.2 Synthetic Alizarin Dye

E-3.3 Methanol

E-3.4 Dichloromethane

NOTE — All reagents shall be of analytical purity

E-4 PROCEDURE

Weigh the two red dyes separately (0.1gm) in 1 000 ml dichloromethane and scan through UV-Visible spectrometer. For visible spectrum this solution is used and for UV spectrum the solution is further diluted by 5 times. Identification of the dye by this method involves an empirical comparison of the details of the spectrum that is, lamda maxima (optical density) of natural *Rubia* and synthetic alizarin red dyes and the characteristic peaks are matched. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm.

E-5 UV CALIBRATION METHOD FOR *RUBIA* DYE DETERMINATION

Calibration curve shall be done by using 2, 4, 6, 8 and 10 mg of *Rubia* dye per ml of methanol The solutions need to be filtered through Whatmann filter paper 42 and then used for UV calibration method and the absorbance shall be measured at 430 nm as shown in Fig. 10.

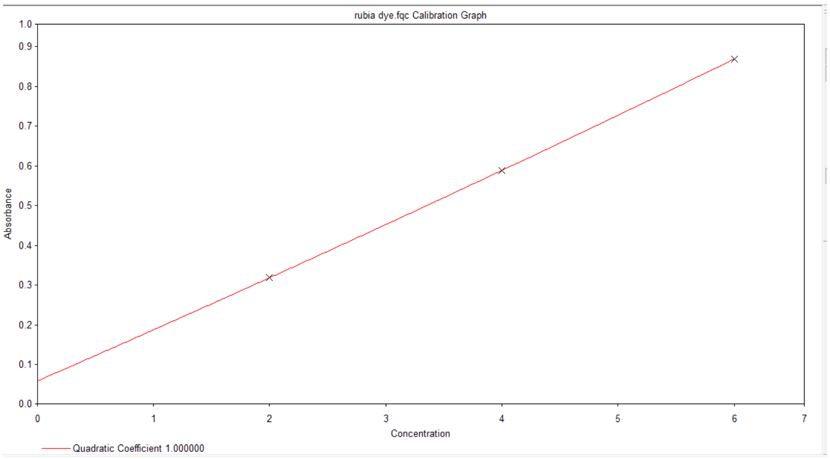

FIG. 10 CALIBRATION CURVE FOR THE *RUBIA* DYE

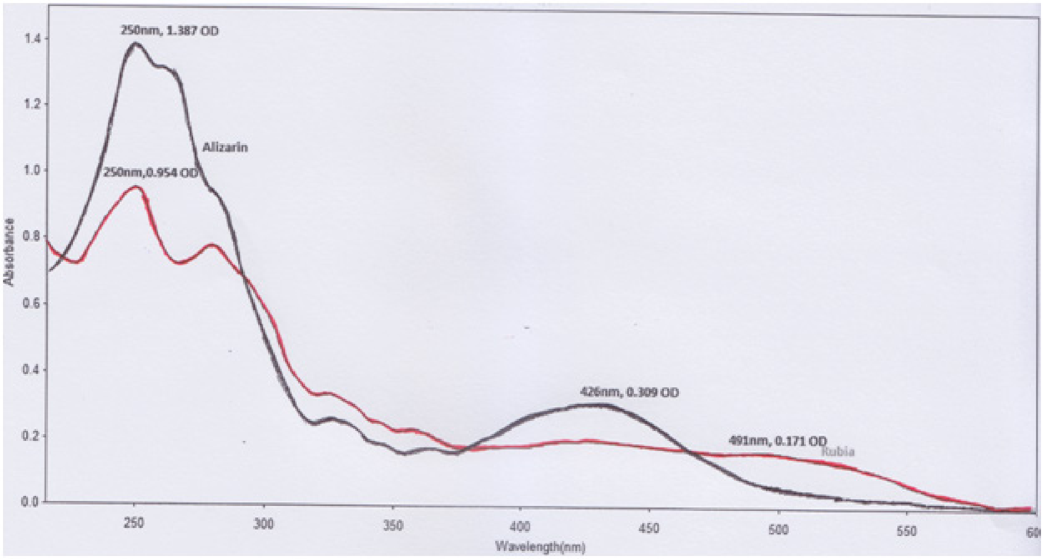

FIG. 11 UV SPECTRUM OF RUBIA AND ALIZARIN DYES

E-6 OBSERVATION

Peak Reading Received at (nm)	**In Synthetic Red Alizarin**	**In Natural *Rubia***	**Comment** **(Describing Difference with Reason)**
	250 nm (1.38 OD) and 426 nm (0.309 OD)	250 nm (0.954 OD) and 491 nm (0.171 OD)	The pattern of the peaks in UV and Visible region are very different for the two dye samples as shown in Fig. 11.

E-7 RESULT

Lamda maxima (optical density) of natural *Rubia* and synthetic alizarin dyes are different. In the UV spectrum, although the peaks are at 250 nm but with different optical densities (OD). In the visible region peaks at 426 nm and 491 nm for alizarin and natural *Rubia* dyes are the characteristic peaks mentioned as shown in the spectra herein.

ANNEX F

(*Clause* 1 *and* 5.3)

SPECTROSCOPIC ANALYSIS OF NATURAL *RUBIA* DYE BY FTIR

F-1 PRINCIPLE

The Infrared spectroscopy (IR Spectroscopy) is the subset of absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify a compound and to investigate the composition of a sample. A beam of infra-red light is produced and split into two separate beams. One is passed through the sample, the other passed through a reference which is often the substance the sample is dissolved in. The beams are both reflected back towards a detector, however first they pass through a splitter which quickly alternates which of the two beams enters the detector.

F-2 APPARATUS

F-2.1 Fourier Transform Infrared Spectrophotometer Machine

F-2.2 Pellet Press Machine

F-2.3 Electronic Balance, Resolution at 0.001g

F-2.4 Spatula

F-2.5 Oven

F-3 REAGENT

F-3.1 Potassium Bromide (Dry)

F-3.2 Dichloromethane (Dry)

F-3.3 Natural Rubia Dye

F-3.4 Synthetic Alizarin Dye

F-4 SAMPLE PREPARATION

Samples for FTIR can be prepared in the following way: Solid samples of natural *Rubia* and synthetic red (alizarin) dyes are milled with dry potassium bromide (KBr) to form very fine powder mixture. Take a small amount of powder sample (about of 0.1-2 percent of the KBr amount, or just enough to cover the tip of spatula) mix with the KBr powder. Subsequently, grind the mixture for 3-5 min. This powder is then compressed into a thin pellet which can be analyzed. KBr is also transparent in the IR.

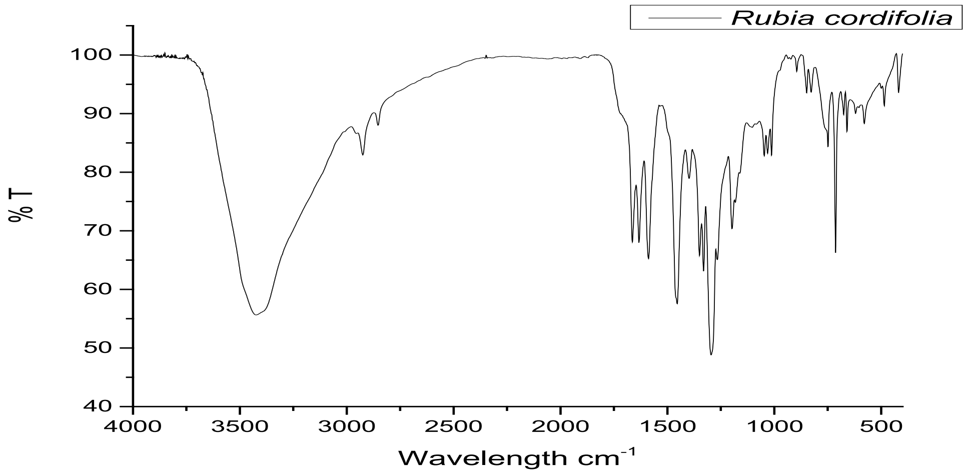

FIG. 12 FT IR SPECTRUM OF RUBIA DYE

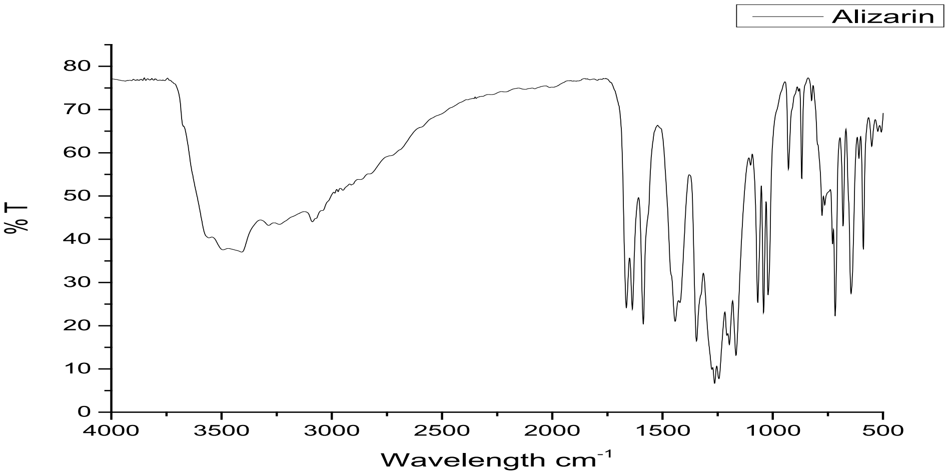

FIG. 13 FT IT SPECTRUM OF SYNTHETIC ALIZARIN DYE

F-5 OBSERVATION

Peak Reading Received at (cm^{-1})	In Synthetic Alizarin Red	In Natural *Rubia*	Comment (Describing Difference with Reason)
	3573, 3403, 2948, 1661, 1636, 1585, 1441, 1346, 1243, 1197, 1067, 927, 772 and 681 cm^{-1}	3379, 1710, 1664, 1631, 1589, 1453, 1330, 1293, 1196, 1012, 894, 827, 675 cm^{-1}	Peaks in *Rubia* are distinctly different due to presence of purpurin As shown in Fig. 12 and 13.

F-6 RESULT

The testing procedure confirm that the product showing that broad peak at 3379 represent natural *Rubia* and is characteristic peak of natural *Rubia*. Sharp peaks at 1710 and 1664 (cm^{-1}) are always present in natural *Rubia* sample.

ANNEX G

(*Clause* 1 *and* 5.3)

EXTRACTION OF NATURAL RUBIA DYE FROM DYED SWATCHES

G-1 PRINCIPLE

The solvent (in soxhlet) extraction of dyed samples is necessary to release the dyes from the dyed fabric samples.

G-2 PROCEDURE

The solvent (in soxhlet) extraction of dyed samples is necessary to release the dyes from the dyed cotton samples. 50 gm of dyed cotton samples from natural *Rubia* and synthetic alizarin red dye are separately cut into small pieces and packed in a filter paper as a filter paper column. The packed filter paper column is put for soxhlet extraction for 6-8 hwith methanol as solvent for extraction, temperature is set at 60°C, till all the color is discharged. Remove the solvent in a vacuum rotatory evaporator to dryness.

G-3 OBSERVATION

The sample is now ready for used for chromatographic and spectroscopic analysis as per the methods described in Annex C-F.

ANNEX H

(*Foreword*)

COMMITTEE COMPOSITION

Textile Speciality Chemicals and Dyestuffs Sectional Committee, TXD 07

Organization	*Representative(s)*
Department for Jute and Fibre Technology Institute of Jute Technology University of Calcutta	Prof A. K. Samanta (***Chairman***)
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Office of the Textile Commissioner Mumbai	Shri B. B. Bharti Shri M. Balasubramanian (*Alternate*)
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U P Textile Technology Institute, Kanpur	Prof Abha Bhargava Dr Arun Patra (*Alternate*)

Organization	*Representative(s)*
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Wool Research Association, Thane	Smt Smita Bait Shri Chetan Mahajan (*Alternate*)
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